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THE ACTIVITY OF SOME ENZYMES IN THE AORTA OF THYMECTOMIZED AND SPLENECTOMISED NEWBORN RATS

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According to data submitted by Lojda(1961), 5'nucleotidase and adenylpyrophosphatase (adenosine-triphosphatase) are established in atheromatous plaques in substantially lower concentration (proved both biochemically and histochemically) than in normal regions of the aorta in rabbits with induced atherosclerosis. These findings are in compliance with the data reported by Kirk (1964), who established a rised activity of both enzymes in women as compared to males and explained the lower morbidity rate of atherosclerosis in females with the enhanced activity of these particular enzymes. Reis (1951) and Zemplenyi (1962) against the background of personal experience and literature data, point out that the activity of 5'nucleotidase is conditioned by tissue calcification.

The surge of interest on the thymus, observed in recent years, enabled the getting acquainted with numerous new facts. Not merely its role has been demonstrated as a basic regulatory factor inasmuch immunologic reactions are concerned (Miller, 1963; Yankovich, 1962; Miller, 1963 a etc.), but its participation in certain carbohydrate metabolism processes as well (Pansky and assoc., 1965). According to data published by Comsa (1959), the thymectomy accounts for a fall of the cholesterol blood content, whereas personal investigations by the authors of the paper, prove that it accounts also for a lowering of the cholesterol level in the aortic wall. Proceeding from the data thus outlined, we undertook the task to verify whether the lipolytic activity in thymectomy is effected by altering the enzymatic content in the aortic wall. Besides that, it is equally interesting to know if this effect might be obtained after the removal of a secondary lymphatic organ such as the spleen.

Material and Method

The experiments were performed on a series of 48 white unbreeded rats, distributed in three groups as follows: first group — comprises 16 rats for control purposes, kept in identical leaving conditions as the test animals ever since the very first day of life; second group of 22 rats — subjected to thymectomy postnatally and third group of 10 rats — subjected to postnatal splenectomy. It should be stressed moreover, that postnatal thymectomy accounts for a high percentage of mortality due mainly to the cannibalism, developing in mothers after the operative intervention upon their offspring. The mortality rate in our experiments does not exceed that reported by other writers. A considerably higher mortality was observed among the splenectomized animals: three fourths of the total number of operated animals died. It is evident that splenectomy proves to be a heavy operation which,

regardless of the meticulous sterility observed, accounted for a high incidence of inflammatory complications in the peritoneum. Owing to the difficulties encountered, the number of splenectomized animals is smaller as compared to the remainder.

The experiment lasted 70 days with sacrification of the animals of each group carried out at pre-established time intervals. In order to trace the process in dynamics, a third of the animals of each group were killed on the 30th, 50th and 70th day of life. Killing was made by exsanguination with ensuing dissection and removal of the entire aorta. After adequate cleansing of the periaortic tissue and washing up with cold saline physiologic solution, the aorta was furthermore homogenized in physiological saline solution and the extract was used for investigation of its enzymatic activity. The Ahmed and Reis method was resorted to for determination of the 5'nucleotidase activity (1958). The adenosine triphosphatase activity was determined according to the method of Bangaa and Novotny (1948) as modified by Zemplenyi and assoc. (1962). Determination of the nitrogen in proteins was made after Lowry (1951).

Results and Discussion

The 5'nucleotidase activity traced up in its various age-group aspects, displayed differences, substantiated by the type of treatment applied to the animals, on one hand, and by their age, on the other (Diagrams I, II, III). The rise of enzymatic activity with the increase of age is characteristic for the control animals. With the latter group the activity is lowest at the age of 30 days, at the age of 50 it is higher and in 70-day-old rats it is increased $2^1/_2$ times as compared to the initial level. This finding shows that the metabolic processes in the aortic wall undergo modifications along with the growth of the organism. The high activity of the enzyme established at the end of the hird month of life, period at which presumably maturity occurs in rats, is maintained at the same level. The data published by Zemplenyi and assoc. (1954) corroborate the latter statement. The activity of these enzymes in thymectomized animals exhibit a phasic nature, parallel to the process of aging. With this group it is worth mentioning that as early as on the 30 th day of life a high activity is established, very similar to that in the adult animals of the control group. At the age of 50 days a substantial fall is recorded (Diagram 1), whereas with reaching adulthood a secondary rise of the activity takes place approximating the level of the control animals of the same age-group. The data presented show that postnatally performed thymectomy brings about changes in the enzymatic content of the aortic wall, corresponding to adulthood. It is rather difficult to offer an explanation of the activity decrease in the 50-day-old animals. Perhaps, it is determined by the period of development of the so-called wasting disease. A general fall of trophic processes is characteristic for the latter condition (Ccmsa, 1959; Miller, 1963) and possibly, the same holds true for the ferment processes within the acrtic wall. In splenectomized animals at the age of 30 days, a high activity is also established of the 5'nucleotidase, corresponding to the mature age. The characteristic features of the changes in the enzymic activity of this particular group consists in the substantial activity

rise at the age of 50 days, quite different from that noted in the control group. The rise just referred to is in discrepancy with the data recorded in the group of neonatally thymectomized and thus, it provides sufficient ground to conjecture that the regularities in the activity alterations of the enzyme studied are different and are conditioned in a variable pattern. It might

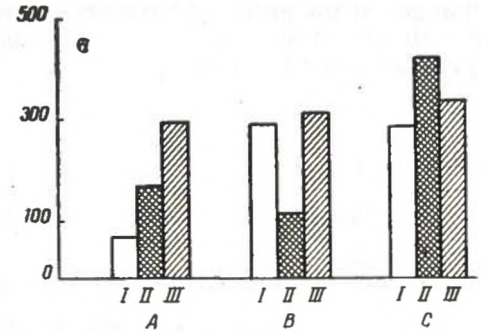

Diagram 1. 5'Nucleotidase activity; a) controls, b) thymectomized and c) splenectomized.
I — 30 days old, II — 50 days and III — 70 days.

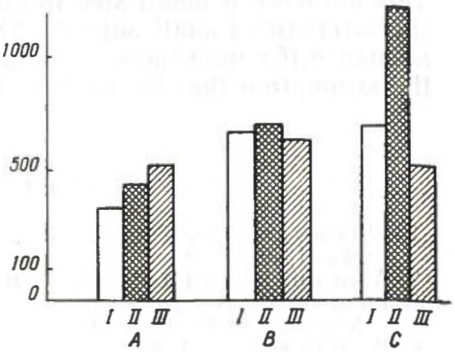

Diagram 2. Adenosinetriphosphatase activity; a) controls, b) thymectomized and c) splenectomized.
I — 30 days old, II — 50 days and III — 70 days.

be assumed that the absence of wasting disease in the neonatally splenectomized animals appears to be the cause for the enzymatic activity difference with this particular group of animals.

The investigation of the adenosinetriphosphatase activity, similarly, displays characteristic features for the individual groups. A tendency towards increase is established in the control animals with growth (Diagram 2). In thymectomized animals, the aging does not account for fluctuations whatsoever in the activity of the same enzyme. It is fixed at a definite level, corresponding to that of adult control animals. In splenectomized animals the activity shows significant fluctuations. At the age of 30 days the activity is high, as in neonatally thymectomized animals. At the age of 50 days, this activity displays nearly twofold rise as compared to the initial level, whilst in adulthood it falls beneath the initial value and is levelled with that of the control group. The data herein reported prove that neonatal thymectomy and neonatal splenectomy alike account for a rise in the activity of adenosine triphosphatase in the aortic wall. The comparative study of the absolute values of enzymatic activity at the age of 50 days in the two groups under investigation shows that the influence is not unidirectional and in all likelihood, appears not to be conditioned by the general functional trends of the two lymphatic organs. The adenosine triphosphatase is an enzyme, specific for the aorta (Balo, Bangaa and Josepovits — 1948, 1949) and its absolute values are the higher, the more resistant the animal species appears to be to atherosclerosis and equally higher the lipolytic activity of the aorta (Zemplenyi and assoc., 1961). Maybe, some of our previously published data, establishing that neonatal thymectomy brings about a reduction of the cholesterol content in the aorta (1966), should be

re-examined and accordingly explained in the light of the newly discovered facts.

In conclusion, it might be stated that both postnatal thymectomy and splenectomy exert an effect upon the activity of the 5'nucleotidase and adenosine triphosphatase in the aortic wall, mainly in younger age-groups. This influence is manifested in premature occurrence of enzymatic activity, characteristic of adult animals. The changes in the group of thymectomized animals differ from those in the group with splenectomy and do not warrant the assumption that the mechanism involved is unidirectional.

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АКТИВНОСТЬ НЕКОТОРЫХ ЭНЗИМОВ В АОРТЕ ПРИ ТИМЭКТОМИИ И СПЛЕНЭКТОМИИ У НОВОРОЖДЕННЫХ КРЫС

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РЕЗЮМЕ

Известно, что тимэктомия вызывает снижение уровня холестерина в сыворотке крови и стенке аорты. Возникает вопрос осуществляется ли липолитическая активность при тимэктомии посредством изменения энзимной активности стенки аорты и приведет ли удаление вторичного лимфатического органа, как селезенки, к аналогичному эффекту.

Путем биохимических исследований, прослежена активность энзимов 5'нуклеотидаза и аденилпирофосфатаза в стенке аорты у неонатально тимэктомированных, неонатально спленэктомированных и у интактных белых крыс, в возрасте 30, 50 и 70 дней.

Результаты показывают, что у контрольных животных, с увеличением возраста нарастает энзимная активность. Воздействие постнатальной тимэктомии и спленэктомии приходится преимущественно на более молодой возраст и выражается в преждевременном установлении энзимной активности на уровне выросших животных. Изменения у тимэктомированных животных являются различными от таковых у спленэктомированных, и не дают основания считать, что их механизм однозначен.